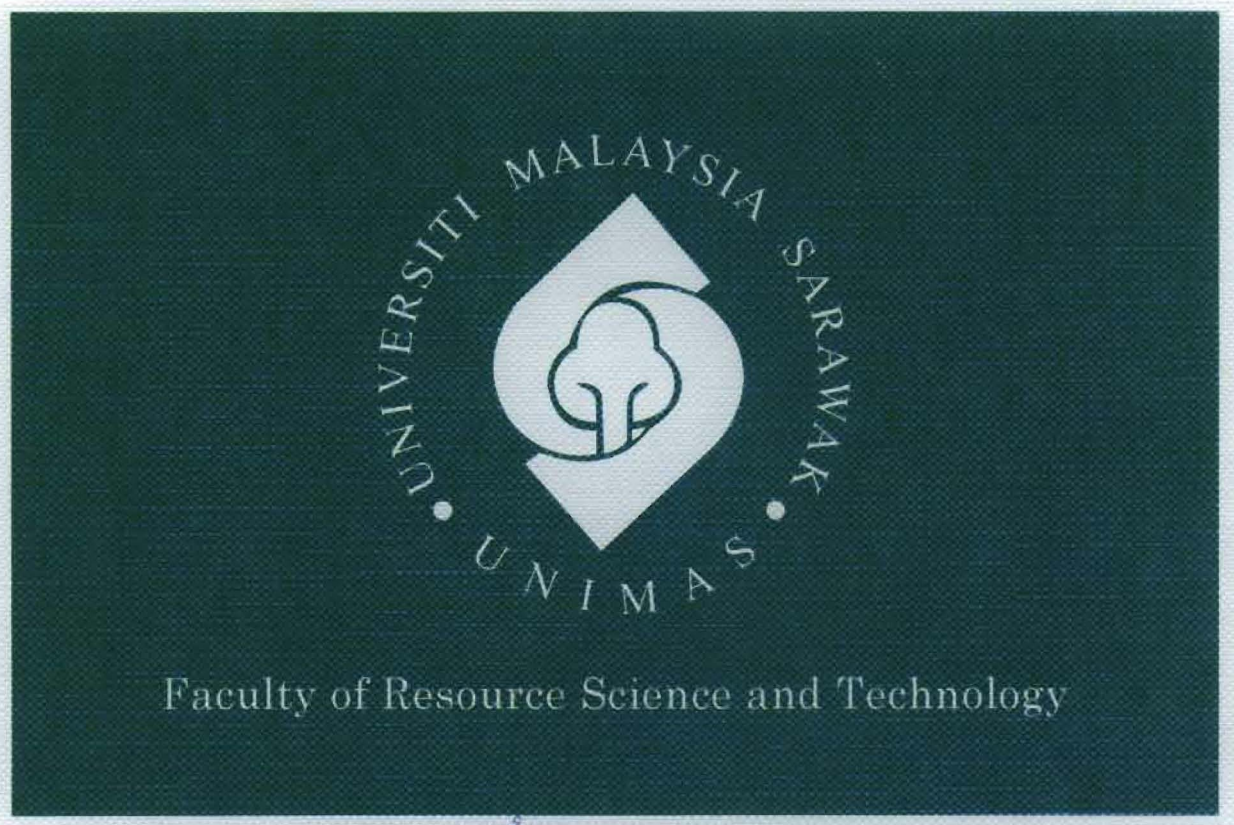

PHYTOPLANKTON COMPOSITION OF SEMATAN ESTUARY, SARAWAK

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Bachelor of Science with Honours
(Aquatic Resource Science and Management)
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Phytoplankton Composition of Sematan Estuary, Sarawak

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20 June 2014

DECLARATION

I hereby certify that this final year project report submitted has no portion of this work has been submitted for the award of any other degree of qualification in any other university or other institution.

_____

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List of Abbreviations

ANOVA	Analysis Variance
ASP	Amnesic Shellfish Poisoning
CFP	Ciguatera Fish Poisoning
DA	Domoic Acid
DO	Dissolved Oxygen
DSP	Diarrhetic Shellfish Poisoning
EDTA	Ethylenediamine - Tetraacetic
H_2SO_4	Sulphuric acid
HAB	Harmful Algal Bloom
$KMnO_4$	Potassium permanganate
$MgCO_3$	Magnesium carbonate
NO_3-N	Nitrate nitrogen
PAST	Paleontological Statistics
pH	Potential Hydrogen
PO_4^{3-}P	Reactive phosphorus
PST	Paralytic Shellfish Tocin
SEM	Scanning Electron Microscope
SiO_2	Silicate
TEM	Transmission Electron Microscope

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Phytoplankton Composition of Sematan estuary, Sarawak

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ABSTRACT

Phytoplankton is an autotrophic component of plankton that drifts in both freshwater and marine environments. Proliferation of some phytoplankton species resulted in Harmful Algal Blooms (HABs). This is a phenomenon which the bloom of toxic or harmful phytoplankton responsible death of aquatic life, destruction of marine ecosystem and seafood unsafe for consumption. The mechanism of this organism to proliferate in the estuarine areas has not been well studied and fully understood. A study was conducted to determine the composition of phytoplankton at Sematan estuary in Lundu, Sarawak. Both qualitative and quantitative phytoplankton samples were collected monthly from September 2013 until March 2014. Fourteen taxa of phytoplankton were identified to generic level and the dominant group was diatoms. The dominance diatoms were *Coscinodiscus* and *Pleurosigma*. Physical parameters showed weak relationship with cell density except for transparency while the macronutrients, all of the parameters have weak impact towards the cell density. There were 6 HABs genera found which were *Ceratium, Chaetoceros, Odontella, Rhizosolenia, Thalassionema* and *Nitzschia*. However, more sampling should be done in order to get more accurate data.

Keywords: Harmful Algal Blooms (HABs), estuary, phytoplankton composition, Sematan

ABTSRAK

Fitoplankton merupakan komponen autotrofik plankton yang hanyut dalam air tawar dan marin. Percambahan beberapa spesies fitoplankton boleh menyebabkan ledakan alga berbahaya (HAB). Fenomena ini bertanggungjawab atas kematian hidupan akuatik, kemusnahan marin dan makanan laut berbahaya untuk dimakan melalui ledakan fitoplankton berbahaya atau bertoksik. Kurang kajian dilakukan terhadap mekanisme organisma ini untuk membiak di kawasan muara dan tidak difahami sepenuhnya. Satu kajian telah dijalankan untuk menentukan komposisi fitoplankton di muara Sematan, Lundu, Sarawak. Sampel kualitatif dan kuantitatif diperolehi setiap bulan dari September 2013 hingga Mac 2014. Fitoplankton telah dikenalpasti ke tahap genus, di mana empat belas taksa fitoplankton telah dikenal pasti dan diatom adalah kumpulan dominan. Diatom yang dominan ialah Coscinodiscus dan Pleurosigma. Parameter fizikal menunjukkan hubungan lemah dengan kepadatan sel kecuali ketelusan cahaya manakala untuk makronutrien, kesemua parameter mempunyai kesan langsung yang lemah terhadap ketumpatan sel. Terdapat 6 genera HAB dijumpai iaitu Ceratium, Chaetoceros, Odontella, Rhizosolenia, Thalassionema dan Nitzschia. Walau bagaimanapun, lebih persampelan perlu dilakukan untuk mendapatkan data yang lebih tepat.

Kata kunci: Ledakan Alga Berbahaya (HAB), muara, komposisi fitoplankton, Sematan

1.0 Introduction

Phytoplankton can be described as the plant of the sea. It has contributes a lot in the world's primary production. Phytoplankton has importance role for forming the basis of food chain. Phytoplankton is a major source of organic carbon at the base in its ecosystem (Laskar & Gupta, 2009; Agboola *et al*., 2011).The phytoplankton is divided into three size classes which are picoplankton, nanoplankton and microplankton. Besides that, the phytoplankton was divided into several groups such as diatoms, dinoflagellates and flagellates. Diatoms had two more division which are the centric diatoms and pennate diatoms.

Phytoplankton is highly sensitive with the environment factor such as physico-chemical and biological. Their sensitivity and large variations in species composition are often a reflection of significance alteration in ambient condition within ecosystem (Adelasu, 2010). The study of phytoplankton community response to environmental variables is considered very useful for interpreting ecological variations amid threats of fishery resource overexploitation, pollution, and climate change (Biswas *et al*. 2010; Kannan & James 2009). Therefore, phytoplankton usually used as biological indicator of pollution at both freshwater and marine.

Since phytoplankton form the base of the marine and freshwater food web, algal blooms have close relationship with the both primary production. The detection and monitoring of these blooms are often considered as a viable means for locating new fishing grounds. Thus, algal bloom monitoring has a positive impact on countries engaged in marine fishing. However, some algal blooms are deleterious to humans or also called Harmful Algal Blooms (HABs). This biological phenomenon give directly and indirectly effects on the human population and also to the ecosystem. Although the phytoplankton is

a small organism and cannot be seen under unaided eye, it still can affect the human population through bioaccumulation and biomagnification of marine toxins. This will affected human through consumption of bivalves and fishes. It also can bring negative impact on marine fisheries and aquaculture.

Estuaries environment has a complex water body which the part of the sea water and freshwater meets. The tolerable organisms that inhabit the estuaries need to have some characteristic that withstand the environmental conditions. Slightly fluctuation in the water quality can make organism like phytoplankton to response. The phytoplankton composition in the water body can be determined at the level of biomass distribution by determined the concentration of chlorophyll. Phytoplankton community and size composition are also known to be related to nutrient availability (Kiorboe, 2001).

Human population is greater at coastal regions. The estuaries and coastline had experience rapid and massive development which can affect the nutrient discharged into the water body. Continuous socio-economic growth and industrialization resulted negative impact to the coastal and estuaries. Concentration of nutrient discharged into the river and sea increasing due to the inclination of the population percentage at the region (Kamaruzzaman *et al*., 2010).

This study was conducted at the Sematan estuary as the study site. It is located at the meeting of small rivers and located at the small town of Sematan. The estuary which also connected with the South China Sea has been the major source for seafood and economy for local people that live near the estuary. Besides that, this area also filled with the industrial area and the tourism places along the river which made potential for algal blooms occur at this area. Therefore, this study was aimed to provide baseline data on phytoplankton composition at the brackish water and the specific objectives are:

1. To determine the phytoplankton composition in the estuary;
2. To determine the effect of selected environmental factors on the phytoplankton composition;
3. To determine the phytoplankton composition pre and during the northeast monsoon;
4. To determine the possible occurrence of HAB species in Sematan.

The monitoring of certain phytoplankton can be aided by the understanding of water quality parameter and importantly its effect on the species composition at certain area. This will be helpful on the certain species especially those who related to the HABs which might occur and give a big threat to economy, ecosystem and most importantly, public health.

2.0 Literature Review

2.1 Types of phytoplankton species in Malaysia waters

There are several types of phytoplankton species that can be found within Malaysia waters such as Bacillariophyta, Cyanophyta and also Dinophyta (Muhammad Adlan *et al*., 2012). According to Shamsudin et al. (1987) report, diatoms are the most abundance phytoplankton found in the coasts of Johore, Kelantan and Terengganu with numerous species such as *Chaetoceros* and *Pleurosigma.* According to Boonyapiwat (1997), the *Chaetoceros compressus* and *Chaetoceros lorenzus* are several types of diatom species that abundant in the Malaysia water body.

Salinity of the water is the main factor of the phytoplankton distribution; the diatoms dominated Langat as well as the Perak estuary (Lassen *et al.*, 2004). The diversity of the phytoplankton also depends on the weather, which in this region the monsoon season will affect the diversity of the phytoplankton in the estuary such as in Perak estuary that freshwater diatom were dominated rather than the marine diatoms. Based on study was done in Pahang estuary by Jalal *et al*. (2011), the most dominant phytoplankton was *Leptocylindrus* sp. and the least found was *Gymnodinium* sp.

2.2 Ecology and distribution of phytoplankton

Determination of composition and distribution of phytoplankton community by using the physiochemical study is very important. These parameters are the factors that contribute in the growth of the phytoplankton. Changes observed in the phytoplankton community can gives impact to the food web structure and energy flow in the pelagic ecosystem (Kiorboe, 2001). Other than that, phytoplankton production and the composition are largely influenced by the concentration of nutrients and the light availability in the

photic zone. Phytoplankton generally utilized certain concentration of nitrate and phosphate (Rahimibashar *et al.*, 2009).

Anthropogenic pollution cannot be excluded from the source of nutrient for phytoplankton growth since the development of eco-social growth and industrialization taken places near the estuary. The enrichment of the water body caused by the rainfall, river runoff and sediment discharge from land drainage also affect the fluctuations of the nutrient concentration in the water body (Hamid *et al*., 2004).

Malaysia experienced monsoon seasons which generally can affect the nutrient in the water body which are the Northeast monsoon and Southwest monsoon. During the Northeast monsoon, area in east coast Malaysia generally received large amount of rainfall starting in November until March (Yoshida *et al*., 2006). Large amount of rainfall can dilute the salinity of the water in particular area. The coastal zones of Malaysia experiences the most intense human activity, where a large percentage of the population, ports, industries, tourism constructions as well as agriculture, aquaculture, fisheries, mineral and oil and gas exploitation, communication, transportation, recreation and sewage discharge result in many conflicting human activities in that region (Kamaruzzaman *et al.*, 2010; Zaleha *et al.*, 2010).

2.3 HAB occurrences in Malaysian water.

The first occurrence of the HAB and shellfish toxicity in Malaysia was reported in 1976 whereby marine dinoflagellate *Pyrodinium bahamense var. compressum* was the main causative agent attributed to the blooms in west coast of Sabah (Roy, 1976). This species showed irregular fluctuations in their populations triggered by nutrient concentration and availability of viable cyst that resulted in unpredictable blooms (Anton *et al*., 2000).

In year 1991, three people had intoxication after consumed mussel collected from mussel farm in Sebatu, Straits of Malacca which caused by the *Alexandrium tamiyavanichii* (Usup *et al*., 2002; Lim *et al*., 2007). Following with the PSP incidence in Tumpat, Kelantan, six persons were hospitalized and one fatal was reported due to consumption on contaminated toxic *Polymeda* sp. or locally known as "lokan" in 2001 that took placed (Lim *et al*., 2004). Later studies by Lim *et al*. (2005) found that *A. taylori* and *A. peruvianum* also occurred in Malaysia waters. However, there were no cases of HAB related to human intoxication and fish mortalities reported in Sarawak (Lim *et al*., 2005).

Other dinoflagellates that were potentially toxic other than were *Dinophysis caudate, D. rotundata* and *Prorocentrum lima* (Usup *et al*., 2002). These type of dinoflagellates attributed in diarrhetic shellfish poisoning (DSP) which is the second important intoxication that occur in high densities. Although the occurrences of *Dinophysis* sp. caused DSP, they never caused fatalities (Lim *et al*., 2004). Ciguatera fish poisoning (CFP) is also one of the seafood poisoning toxins that were produced by benthic dinoflagellates such as *Gambierdiscus toxicus, Ostreopsis ovate* and also *Coolia* spp.

3.0 Materials and Methods

3.1 Study site

This study was conducted at the Sematan estuary (Figure 3.1). This estuary is the place where small rivers met. The samples were collected at the jetty of the Sematan town. This station is selected due to the development and activity that occur at that station. Those activities will result in increase of nutrient concentration in the water body of the estuary and indirectly affect the phytoplankton composition in the Sematan area.

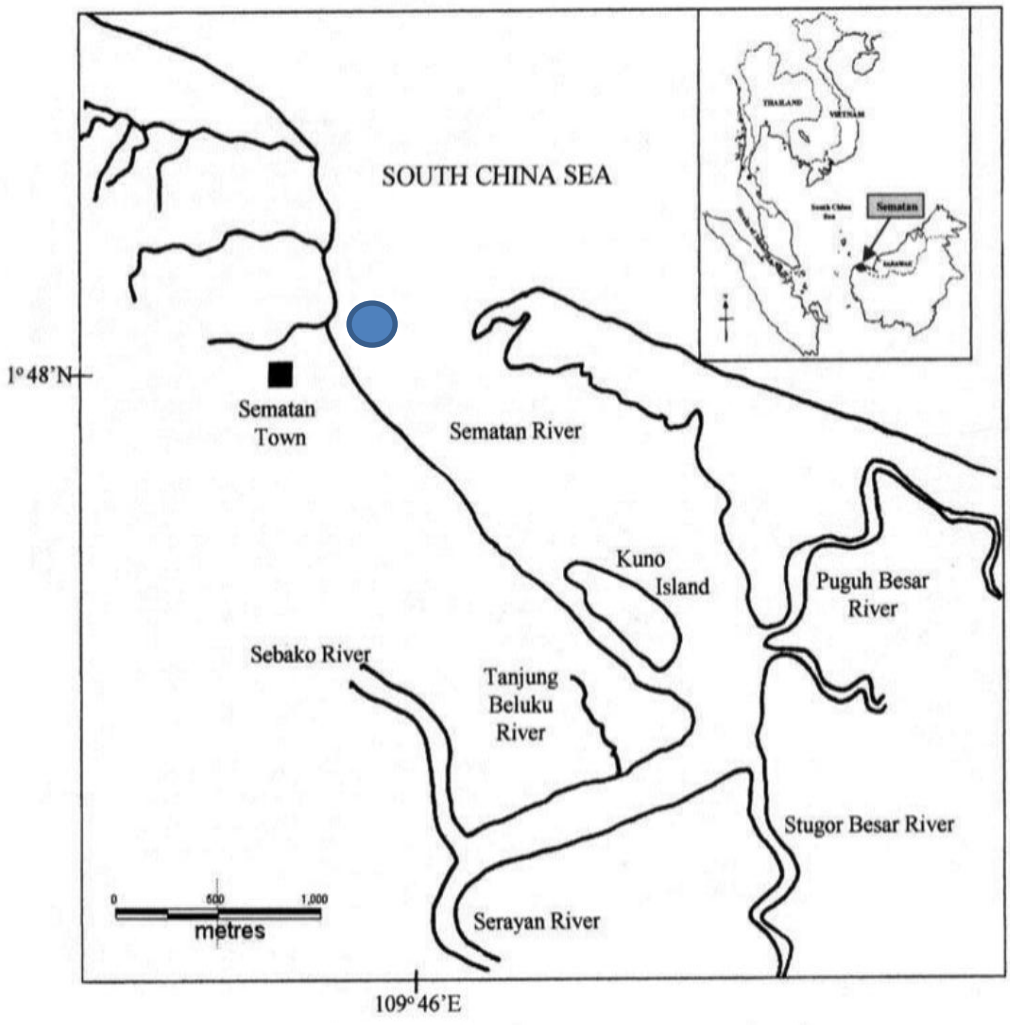

Fig. 3.1: Sampling location at Sematan Estuary. Map modified from (Ikhwanuddin et al., 2012)

3.2 Collection of Phytoplankton and Water Samples

Sampling was carried monthly from September 2013 to March 2014 at Sematan estuary. Qualitative and quantitative samples were collected for phytoplankton and nutrient analysis. Qualitative samples were collected by filtering the water through a 20µm mesh net, while quantitative samples were collected using Van-Dorn water sampler and keep it in 1 litre bottles. The *in-situ* parameters such as temperature, salinity and pH were measured using the HANNA Electronic pH meter and AGATO Hand Refractometer. Transparency was using Secchi disk.

3.3 Phytoplankton Analysis

3.3.1 Cell Counting and Identification

For preparation of cell counting, all concentrated samples were preserved with acidic Lugol's iodine solution and kept under room temperature. Sedgewick-Rafter counting chamber were used under light microscope for cell counting and each sample was counted triplicate. The identification was carried based on the book Identifying Marine Phytoplankton written by Tomas (1997).

3.4 Physical and Chemical Analysis

3.4.1 Chlorophyll *a* Analysis

500mL of water sample were filtered using a 47mm GF/C glass fibre filter. The filtered water then proceeded to the nutrient and water analysis. Chlorophyll *α* retained on the filter paper were used for the chlorophyll *a* analysis. The filter papers were homogenized in 10mL of 90% aqueous acetone. The solution (filter paper and acetone)

then centrifuged and the supernatant after the centrifuging were measured into a 1 cm path length quartz cuvette. The extractions were measured at the following wavelength: 750, 663, 645 and 630 nm. Concentration of the chlorophyll a was calculated using the following formulae:

$$[\text{Chl a}](\mu\text{g L}^{-1}) = \frac{[11.64(Abs_{663}) - 2.16(Abs_{645}) + 0.1(Abs_{630})E/F}{V/L}$$

Where F = dilution factor
E = volume of acetone in mL
V = volume of seawater filtered in L
L = the cell/cuvette path length in cm
Abs_{663} = (reading at 663 nm) - (reading at 750 nm)
Abs_{645} = (reading at 645 nm) - (reading at 750 nm)
Abs_{630} = (reading at 630 nm) - (reading at 750 nm)

3.4.2 Water Filtration and Nutrient Analysis

The filtrate obtained as described previously (Section 3.4.1) were kept under -20°C. Besides that, the *ex-situ* parameters such as nitrate-nitrogen (NO_3-N), reactive phosphorus (PO_4^{3-}-P) and silicate (SiO_2) were determined using portable spectrophotometer HACH Kit DR2800 using colorimetric method (Hach, 2013).

3.5 Data Analysis

Graphpad Prism version 5.0 was used for graph preparation. The correlation between water parameter and cell density was done using the Pearson correlation. The statistical analysis $p = 0.005$ was used.

4.0 Results

Six samplings were carried out from end of September 2013 to March 2014 at the Sematan estuary. From this sampling, 18 bottles of water and plankton samples were collected and analysed for the nutrient analysis, Chlorophyll *a* analysis and cell enumeration.

4.1 Qualitative data: Relative abundance of phytoplankton composition

A total of 14 taxa were identified to the genus level. There were 13 genera of Diatoms which comprised of 3 pennate diatoms and 10 centric diatoms. There was only one genus found for dinoflagellates which is *Ceratium*.

The dinoflagellates *Ceratium* had the highest abundance throughout the 6 sampling months, particularly in September 2013 and January 2014 where its abundance dominated nearly half of the total phytoplankton species, which in September the species abundance is 47.52% and in January is 44.82% (Figure 4.1.1). The second most abundant phytoplankton was *Pleurosigma* spp., especially where its relative abundance was the highest in the November (Figure 4.1.1).

Overall, there were 3 taxa that can be found throughout the sampling periods. The *Pleurosigma* sp. and *Coscinodiscus* sp. was the common diatoms species that occurred on all of the sampling months. While for dinoflagellates, *Ceratium* sp. was the only and common species found on all of the sampling months. The least common species were *Nitzschia* sp. which only occurred in November and *Rhizosolenia* sp. which occurred in September.

4.2 Quantitative data: Total cell density

Total cell density were inconsistent throughout the sampling periods, with the obvious trend resulted by the amount of diatoms (Figure 4.2.2). The total cell density ranged from 39,667cells/L to 336,333cells/L in the sampling periods. The highest amount of phytoplankton occurred was in the month of September where the diatoms were 182,333cells/L and dinoflagellates were 154,000cells/L. The lowest number of phytoplankton was in November where the diatoms were 29,000cells/L and dinoflagellates were 10,666cells/L.

From the total cell density, phytoplankton was grouped into two major group, diatoms and dinoflagellates. Diatoms had outnumbered dinoflagellates in all the sampling periods. Diatoms group recorded about 70.35% of all cells and the remaining was the dinoflagellates.

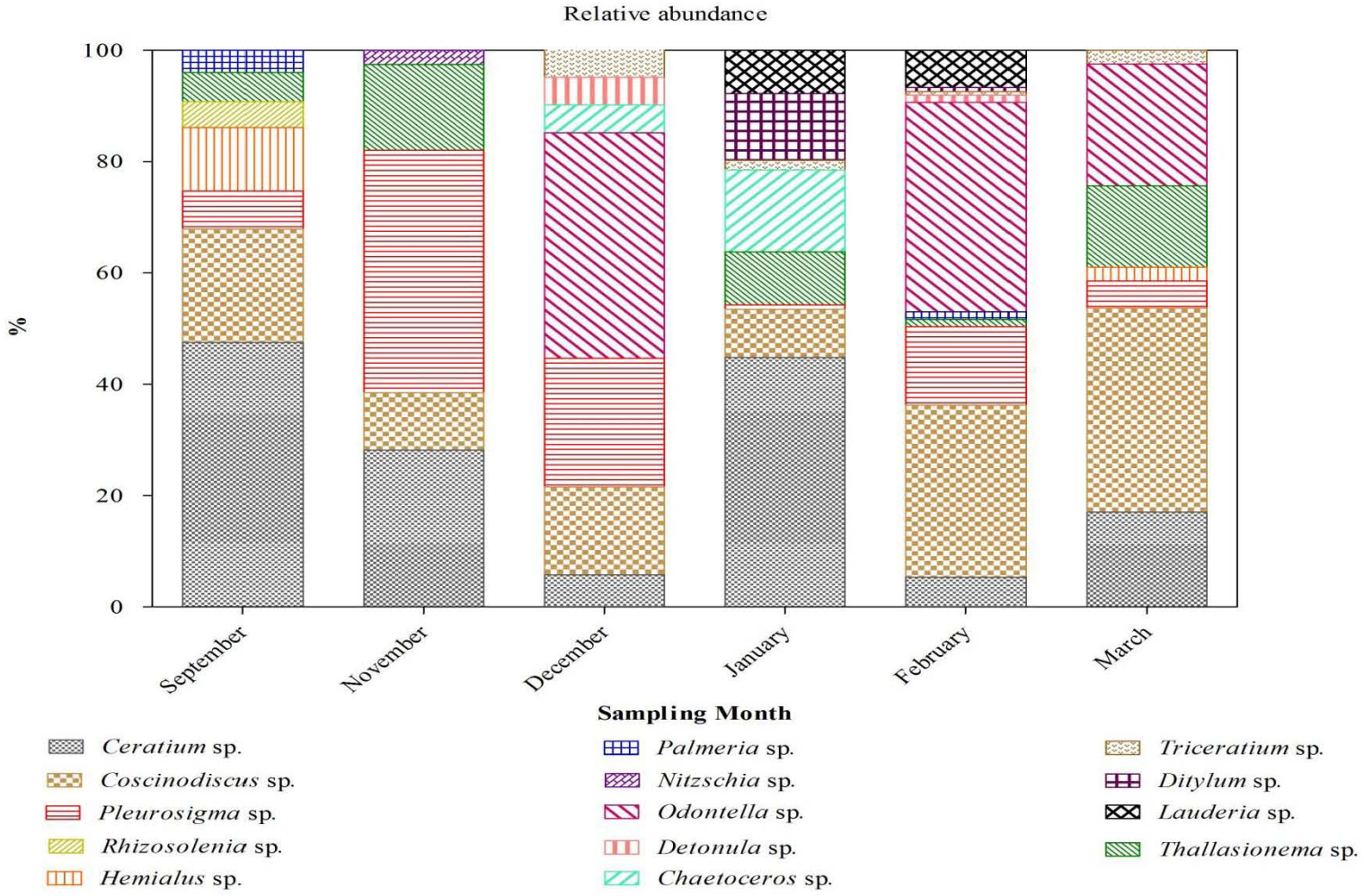

Figure 4.1.1:Relative abundance of various phytoplankton taxa for 6 sampling month ranging from September 2013 to March 2014

Table 4.1: Occurrence of phytoplankton at Sematan estuary.

	Occurrence of phytoplankton					
	2013			2014		
Taxa	28 Sept	14 Nov	5 Dec	12 Jan	25 Feb	4 Mar
DIATOMS (Pennate)						
*Nitzschia**		+				
Pleurosigma	+	+	+	+	+	+
*Thalassionema**	+	+		+	+	+
DIATOMS(Centric)						
*Chaetoceros**			+	+		
Coscinodiscus	+	+	+	+	+	+
Detonula			+		+	
Ditylum				+	+	
Hemiaulus	+					+
Lauderia				+	+	
*Odontella**			+		+	+
Palmeria	+				+	
*Rhizosolenia**	+					
Triceratium			+	+	+	+
DINOFLAGELLATES						
*Ceratium**	+	+	+	+	+	+
TOTAL	7	5	7	8	10	7

*= potentially harmful phytoplankton

Highlighted = phytoplankton that occurred during all sampling period

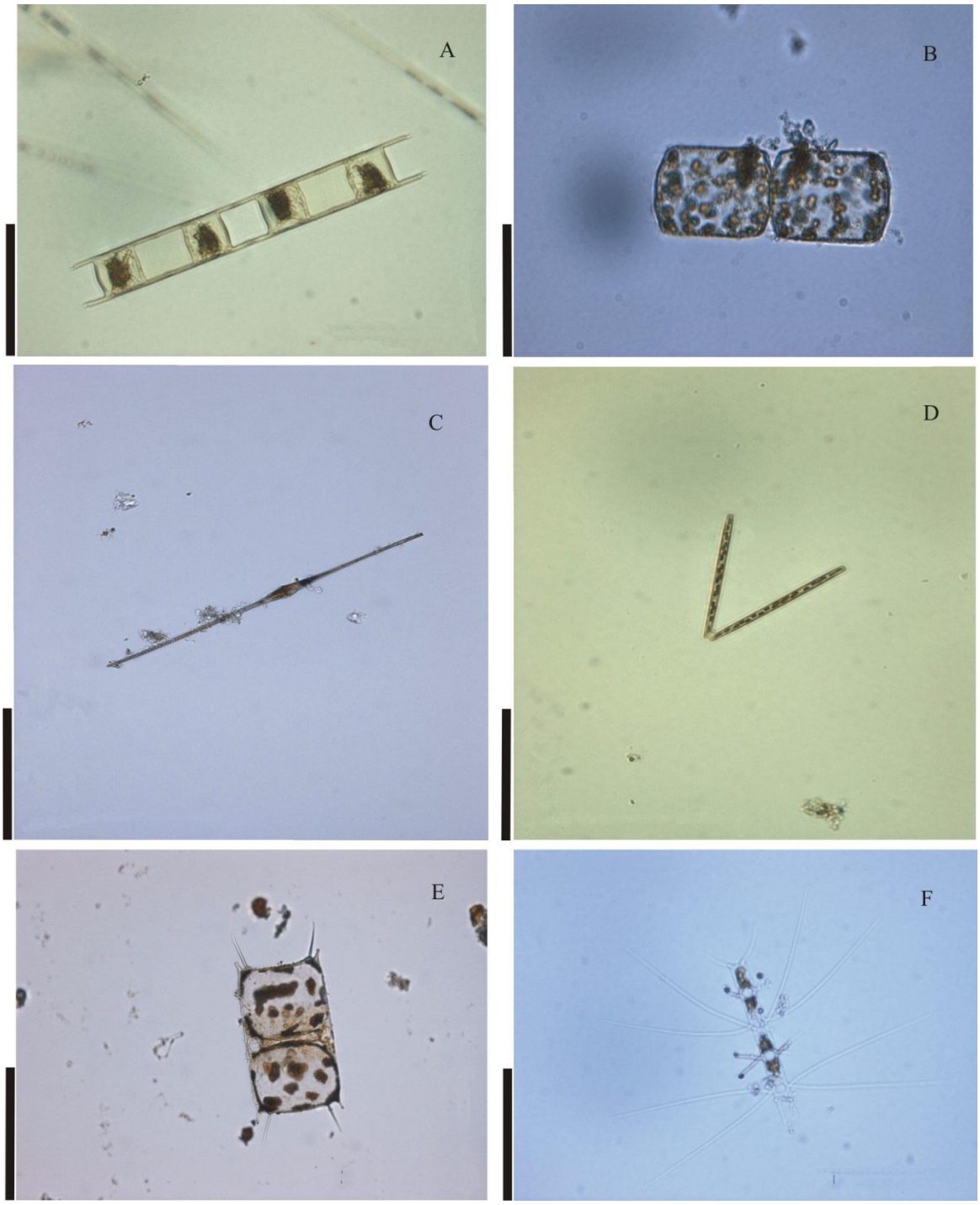

Figure 4.1.2 : Micrograph taken for (A) *Hemiaulus* sp,(B) *Lauderia* sp.,(C) *Ceratium fusus.*,(D) *Thalassionema* sp.,(E) *Odontella* sp., (F) *Chaetoceros* sp. with scale bar=50µm for (B), scale bar = 100µm for (A, D and F), scale bar= 200µm for (C and E).

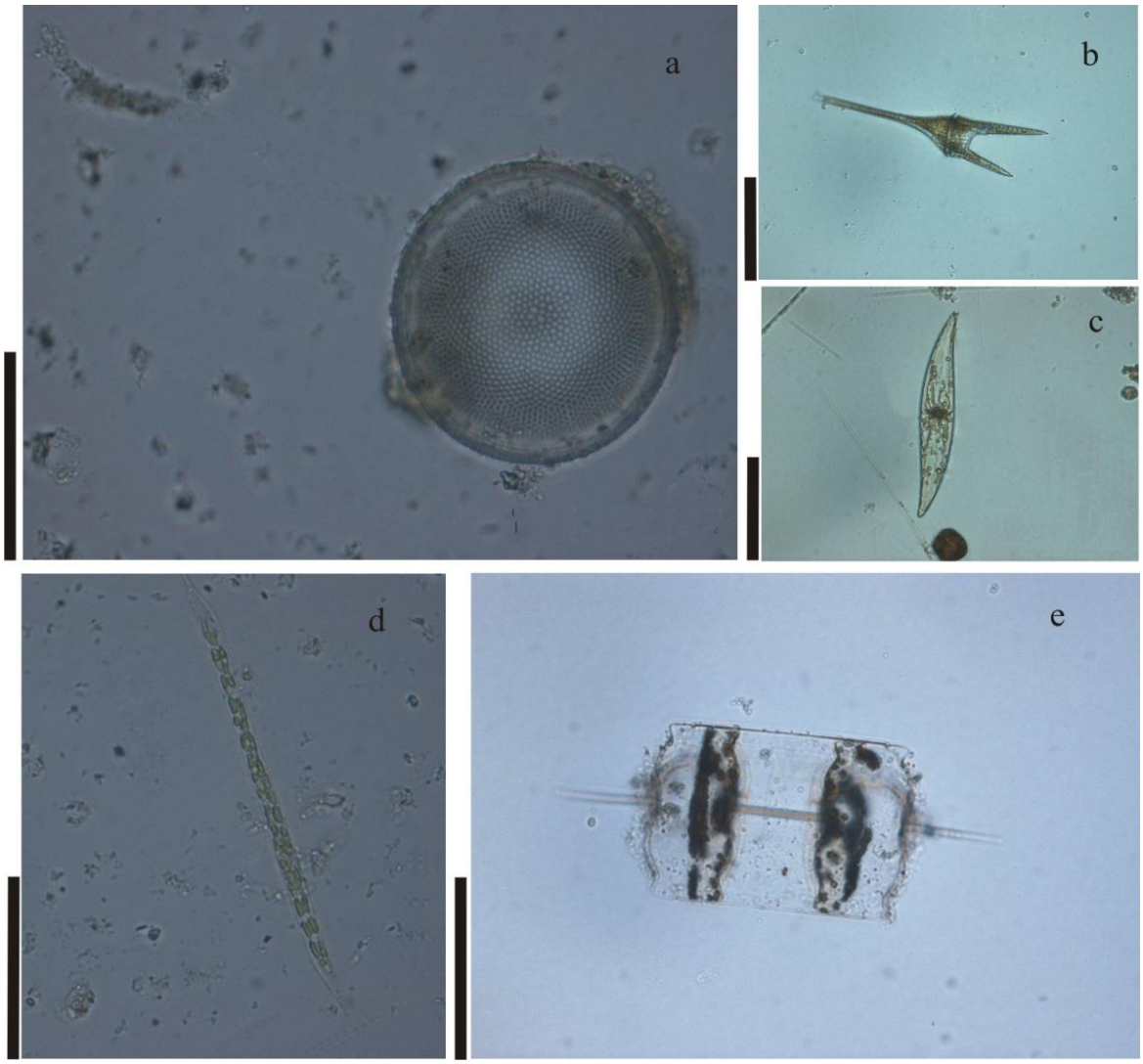

Figure 4.1.3: Micrograph taken for (a) *Coscinodiscus* sp., (b) *Ceratium furca* (c) *Pleurosigma* sp., (d) *Rhizosolenia* sp., and (e) *Ditylum* sp. with the scale bar= 50µm for (a and d), scale bar= 100µm for (b, c and e).